Effects of Putrescine and Spermine on Pollen Germination Levels of Some Olive Cultivars

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Abstract. In this study, it is aimed to determine the effects of putrescine and spermine on pollen germination levels of 8 olive cultivars. Arbequina, Gemlik, Halhalı, Karamani, Kozan yerli, Saurani, Sayfi and Şami olive cultivars were used as material. In the study, 5 spermine doses (0,25mM, 0,125 mM, 0,025 mM, 0,05 mM and 0,005 mM) and 5 putrescine doses (0,20 mM, 0,10 mM, 0,05 mM, 0,025 mM and 0,1 mM) added to basal germination medium (15% sucrose + 1% agar + 100 ppm Boric acid) were tested for determining the effects on pollen germination of olive cultivars. Basal media was used for Control treatment. In terms of pollen germination media, pollen germination levels were increased with lower putrescine and higher spermine levels. In Sayfi olive cultivar, while pollen viability rate was 42,74%, pollen germination was 0,00% in control treatments. However, pollen germination was increased for Sayfi olive cultivar with 0,20 mM and 0,10 mM putrescine (3,64% and 3,40%, respectively) and 0,25 mM, 0,125 mM and 0,05 mM Spermine (12,56%, 4,73% and 3,40%, respectively). In conclusion, polyamine treatments were increased the low germinated cultivars. So, polyamine treatments reveal the potential of the pollen especially in low-germinated pollens.

Keywords: Pollen, Polyamine, Climate change, Quality

INTRODUCTION

Turkey is one of the most important olive producing countries with its 1 739 000 tonnes olive production in 2021 (5). Olive was expanded since 6000 years ago in Mediterranean basin. Turkey has an important place in olive production but has risks in recent years because of climate change which causes destructive effects on flower biology of plants. It affects ovule and pollen fertility which directly influences fruit set level of plants.

Pollen is a unique plant tissue that potentially can be used and manipulated to the advantage of the geneticist, breeders, physiologists or germplasm curators. Pollen quality of olives depends on genetic and environmental factors (7). Especially extreme temperatures have negative effects on pollen germination and tube growth of olive which directly affect fruit set (3; 9).

In olives, like most of the plants, temperature reduction below 12°C and above 30°C reduces the functional pollination due to pollen development failure and low viability. Recent studies showed that polyamines play important roles on signalling process of flower initiation, flower development, blooming periods, pollen germination capacity and ovule longevity. At the same time, polyamines play important roles in temperature balancing of plants (2). Polyamines were also accepted as pollen and pollen tube promoters as it used in proper doses (1) and the compensatory effect of putrescine was reported before on pear (4) and almond (16; 14; 15). Researchers were also reported that while lower doses of putrescine help pollen tube emergence, high doses blocks pollen tube growth powerfully and changes the shape of the pollen tube (1; 15).

The usage of polyamines on olives was limited in previous studies. Constitutively, the exogenous putrescine treatment affects fruit set (13) and this is related with the polyamine content of olive cultivars (12). In this study, it is aimed to determine the effects of putrescine and spermine on pollen germination levels of eight different olive cultivars. So, the usability and the proper dose of polyamines for the negative effects of climate change on olives were aimed to decrease for a better fruit set.

MATERIALS AND METHODS

Materials

This study is conducted on 2023 olive flowering period. The materials were obtained from the olive orchard planted in Cukurova University, Kozan Vocational High School and the pollen tests were carried out in the Cytology Laboratory of Cukurova University Department of Horticulture. In the study; Arbequina, Gemlik, Halhalı, Karamani, Kozan yerli, Saurani, Sayfi and Şami olive cultivars were used to

determine the effects of putrescine and spermine on pollen germination levels of olives. The olive trees were at 8 years old and planted at 6x6 m intervals.

Spermine and Putrescine were used for polyamine sources to obtain different germination media. 5 doses of spermine and 5 doses of putrescine were added to the germination media.

Methods

Pollens were obtained in full flowering period by placing oily papers on branches before pollen releasing and the pollens were taken by shaking these branches. The collected fresh pollens were immediately brought to the laboratory for pollen viability and germination tests.

Determining Pollen Viability Rate

Pollen viability rates were determined using 1% TTC test (2,3,5 Triphenyl tetrazolium chlorid). TTC was prepared and evaluated according to Norton (1966). For each genotype 3 slide replication were prepared and at least 100 pollen grains were counted from each replication (Figure 1). Pollen counting were made by Olympus BX51 microscope. While counting, red pollens were considered as "viable", light pinkies as "semi viable" and uncoloured pollens as "non-viable" (Figure 1). Besides the viable, semi-viable and non-viable pollen ratios, the pollen viability rate was also calculated according to Norton (1966) as the ratio of "sum of total viable and half of semi viable pollens" to "total pollen number". Pollen viability level was determined for controlling the pollen quality.

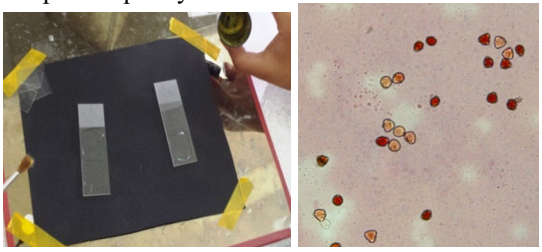

Figure 1. Conducting pollen viability tests.

Determining Pollen Germination Rate and Effect of Polyamine Levels

In vitro pollen germination tests were determined using agar in petri method with spermine (0,25mM, 0,125 mM, 0,025 mM, 0,05 mM and 0,005 mM) and putrescine (0,20 mM, 0,10 mM, 0,05 mM, 0,025 mM and 0,1 mM) doses added to the basal olive germination medium including 100 ppm Boric acid, 1% agar and 15% sucrose (8). Putrescine and Spermine doses were considered according to Sorkheh et al. (2011) and the basal medium was used for Control treatments.

Media and germination petri dishes were prepared according to Dölek-Gencer et al. (2023). Pollen germination ratio for each medium was determined after 24 hours. The pollen grains were considered to be germinated when the pollen tube length was greater than the diameter of the pollen grain (Figure 2). At least 100 pollen grains were counted for each replication.

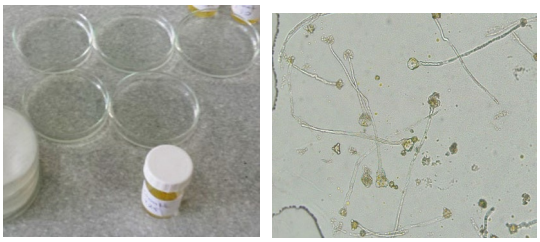

Figure 2. Conducting pollen germination tests.

Statistical Analysis

All data analysis was performed using JMP 13 statistical software. The effects of the treatments were analysed using one way anova analysis of variance. A P value of < 0,05 was considered to be significant. While pollen viability data were conducted in terms of only cultivars, pollen germination data was conducted in terms of cultivars and germination media (doses) separately for each polyamine sources. By this way, 2 factorial randomised design has been used for each polyamine. Percentages were analysed after arc-sin transformation.

In vitro Pollen Viability Level

The pollen viability level has been shown in Table 1. The table showed viable, semi-viable, non-viable and the viability levels of 8 olive cultivars. The viable pollen data were statistically important and the highest viable pollen was obtained from Şami (58,66%) while Halhalı (65,53%) and Gemlik (60,28%) were at the same group with Şami. The lowest viable pollen rates were obtained from Arbequina (11,94%), Karamani (17,04%) and Sayfi (20,95%). The semi-viable pollen ratio were statistically not important and differed between 21,49 % (Halhalı) and 43,58% (Sayfi). The non-viable pollen ratio was significantly different between cultivars. The lowest non-viable pollen ratio was in Şami (7,28%) and the highest in Arbequina (48,72%).

In terms of pollen viability, the data showed that cultivar averages showed statistically important differences. The highest data obtained from Şami, Halhalı and Gemlik cultivars respectively (80,69%, 76,28% and 75,96%) while the lowest obtained from Karamani (34,31%) and Arbequina (31,61%) olive cultivars.

In recent studies Palascino et al. (2008) have been reported that pollen viability was differed between 48,0% (Cellina di Nardo) and 97,6% (Cipressino). In same ecological conditions, Dölek-Gencer et al. (2023) have been reported the pollen viability level between 77,41% (Sarı Ulak) and 81,51% (Gemlik) cultivars. Our results showed parallel data with recent studies.

Table 1. Pollen viability levels of used olive cultivars[1].

Cultivars	Viable (A)	Semi-viable (B)	Non-viable (C)	Viability (A+B)/2
Arbequina	11,94 c[2]	39,34	48,72 a	31,61 d
Gemlik	60,28 a	31,35	8,37 d	75,96 a
Halhalı	65,53 a	21,49	12,98 cd	76,28 a
Karamani	17,04 c	34,54	48,41 a	34,31 d
Kozan yerli	52,88 ab	35,22	11,90 cd	70,49 ab
Saurani	40,54 b	33,77	25,69 bc	57,42 bc
Sayfi	20,95 c	43,58	35,47 ab	42,74 cd
Şami	68,66 a	24,06	7,28 d	80,69 a
$P_{0,05}$	***	N.S.	***	***

[1] Statistical analysis were made after arc-sin transformation.
[2] Differences between averages showed by different letters are statistically significant
N.S. means not-significant; *** means p<0,001.

In vitro Pollen Germination Levels

The results of *in vitro* pollen germination levels in terms of putrescine including media were given in Table 2. The cultivar averages were found to be important at p<0,001. In terms of cultivar averages the highest pollen germination level was determined in Gemlik cultivar with 47,41% while the lowest level was found to be in Sayfi cultivar (1,24%) which has one of the highest non-viable pollen ratio. The *in vitro* pollen germination levels were significantly affected from putrescine doses and lower doses has better results in terms of doses averages.

However, the cultivar x doses interactions have been showed interesting results. In Sayfi cultivar there was not any pollen germination in Control treatment and in 0,10 mM and 0,20 mM putrescine including media the pollen germination could be induced. Similar situation was seen in Saurani that has only 8,67% germination level in Control treatment. In spite of this, in the cultivars which has high germination capacities (Arbequina, Gemlik, and Halhalı), the pollen germination was decreased with increasing putrescine treatments (Table 2).

Table 2. *In vitro* pollen germination level of 8 cultivars in different putrescine doses[1].

Cultivars	**Medium (mM)**						**Cultivars average**
	0,20	**0,10**	**0,05**	**0,025**	**0,01**	**0**	
Arbequina	22,06 m-p[2]	23,49 l-p	28,57 h-o	34,48 f-m	40,95 b-h	40,67 b-h	31,70 C
Gemlik	28,84 h-o	41,67 b-h	50,73 a-d	56,02 a	52,89 abc	54,33 ab	47,41 A
Halhalı	24,16 k-p	38,28 d-j	39,66 c-ı	43,76 a-g	48,89 a-e	53,33 abc	41,35 B
Karamani	15,99 pqr	27,50 ı-p	35,37 e-l	36,76 e-k	43,74 a-g	30,67 g-o	31,67 C
Kozan yerli	30,79 g-o	26,47 j-p	38,10 d-j	19,73 opq	32,81 f-n	38,67 d-j	31,09 C
Saurani	26,72 j-p	9,88 qr	34,20 f-m	20,67 nop	16,40 pqr	8,67 rs	19,42 D
Sayfi	3,64 st	3,40 t	0,00 t	0,00 t	0,00 t	0,00 t	1,24 E
Şami	20,74 nop	24,11 k-p	36,15 e-l	31,51 f-n	44,32 a-f	34,06 f-l	31,81 C
Media av.	21,62 *B*	24,35 *B*	32,86 *A*	30,38 *A*	35,01 *A*	32,56 *A*	
$P_{0,05}$	P_{doses}: *** $P_{cultivars}$: *** $P_{dosesxcultivars}$: ***						

[1] Statistical analysis were made after arc-sin transformation.
[2] Differences between averages showed by different letters are statistically significant
*** means p<0,001.

The *in vitro* pollen germination levels of 8 cultivars at spermine including media have been shown in Table 3. The table shows that pollen germination levels were affected from cultivars, doses and cultivar x doses interaction. The average pollen germination level was higher than 30% except from Sayfi (3,44%) and Saurani (19,42%) and the highest pollen germination was obtained from Halhalı (52,06%) olive cultivar. The spermine treatment averages were generally lower than Control

(32,32%) treatment while it was higher in 0,005 mM (32,73%) and 0,125 mM (33,95%) spermine including media.

The cultivar x doses interaction showed different results (Table 3). In terms of interaction pollen germination was higher especially in low pollen germination capacity cultivars like Sayfi (0,00%) and Saurani (8,64%). At the same time spermine promoted the germination level of Halhalı, Karamani and Şami olive cultivars. Same situation was seen in Table 2 for putrescine treatments.

Table 3. *In vitro* pollen germination level of 8 cultivars in different spermine doses.

Cultivars	**Medium (mM)**						**Cultivar averages**
	0,25	**0,125**	**0,05**	**0,025**	**0,005**	**0**	
Arbequina	10,85 qr	12,70 opq	11,67 pq	18,12 nop	37,50 e-k	39,79 c-j	21,77 D
Gemlik	32,67 jkl	51,29 abc	38,65 d-k	45,19 a-ı	40,81 b-j	53,88 a	43,75 B
Halhalı	41,06 b-j	57,09 a	55,14 a	50,57 a-d	55,73 a	52,78 ab	52,06 A
Karamani	45,90 a-h	38,10 e-k	35,31 g-k	33,59 ıjk	27,45 k-n	30,76 j-m	35,18 C
Kozan yerli	21,02mno	30,06 j-m	47,64 a-f	32,12 jkl	46,50 a-g	38,35 e-k	35,95 C
Saurani	18,55 nop	41,65 b-j	18,44 nop	19,01 nop	22,31 lmn	8,64 qr	21,43 D
Sayfi	12,56 pq	4,73 rs	3,40 st	0,00 t	0,00 t	0,00 t	3,44 E
Şami	47,89 a-e	35,96 f-k	34,62 h-k	41,25 b-j	31,42 j-m	34,78 g-k	37,65 C
Medium Av.	28,77 *B*	33,95 *A*	30,61 *B*	29,99 *B*	32,73 *AB*	32,38 *AB*	
$P_{0,05}$	P_{doses}: *** $P_{cultivars}$: *** $P_{dosesxcultivars}$: ***						

[1] Statistical analysis were made after arc-sin transformation.
[2] Differences between averages showed by different letters are statistically significant
*** means p<0,001.

In recent studies, the *in vitro* pollen germination capacity differed between 12% and 60% (6). The pollen viability and germination levels affect from genetic and environmental conditions and especially the high temperatures like 30-35°C cause a distinct decrease in pollen germination level (3 and 9). In order to prevent the decrease at negative effects of pollen quality, Sorkheh et al. (2011) conducted a study in almonds and found that pollen germination and tube growth promoted in extreme temperatures (10°C or 35°C) with putrescine, spermine and spermidine including germination media. We also found that in cultivars that has low pollen quality, pollen germination could be increased with exogenous putrescine and spermine treatments.

CONCLUSION

In conclusion, pollen germination capacity was significantly influenced from cultivars but not have much significant effects in terms of polyamine treatments. However, polyamine treatments were increased the low germinated cultivars. So, polyamine treatments reveal the potential of the pollen especially in low-germinated pollens.

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